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Concurrent Presentation of Erythrocytic and Megakaryocytic Aplasia

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A case of a patient presenting with idiopathic concurrent erythrocytic and megakaryocytic aplasia is reported. The patient's response to immunosuppressive therapy and her bone marrow pathology clearly suggest an immune mechanism. Based on the lack of suppression of erythroid colony growth, several mechanisms are postulated. Well-established molecular and genetic evidence, along with clinical observations, suggests that a relationship exists between the erythrocytic and megakaryocytic cell lines. This may be related to a common bipotential stem cell or common cell surface markers. This case provides strong clinical evidence to support this relationship. © 1996 Wiley-Liss, Inc.

Key words: red cell aplasia, amegakaryocytic thrombocytopenia, anemia, hematopoiesis

INTRODUCTION

A generally accepted model of hematopoiesis postulates that multipotential stem cells with the capabilities of replicating and generating more differentiated daughter cells give rise to pluripotential stem cells. These cells include the pluripotential lymphatic stem cell and the pluripotential hematopoietic cell. These cells, in turn, give rise to more committed stem cells that generate morphologically distinctive progeny. Human pluripotential hematopoietic stem cells are referred to as colony-forming units-granulocyte, erythroid, monocyte, and megakaryocyte (CFU-GEMM). Such cells may give rise to more restricted (unipotential) progenitors specific for their respective cell lines. These are CFU-erythroid (E), CFU-megakaryocytic (meg), and CFU-granulocyte, monocyte (GM) [1].

Recent evidence (based on a stem cell competition hypothesis) suggests that megakaryocytic and erythrocytic cell lines may share a common bipotential stem cell [2]. Also, erythrocytic and megakaryocytic precursors may have molecular and genetic similarities [3,4].

We present a unique case of a 32-year-old woman with pure red cell aplasia and superimposed amegakaryocytic thrombocytopenia with no clear exogenous etiology. This case supports the existence of a bipotential common precursor of megakaryocytic and erythrocytic lineages or a target antigen for autoreactive lymphocytes common to their respective precursor cells.

CASE HISTORY

J.H. is a 32-year-old woman who presented to another hospital in October 1992 with anemia. The complete blood count (CBC) showed: white blood cell (WBC) 4,500/mm^3, Hgb 6.0 gm%, platelets 255,000/mm^3, and reticulocyte count 0.7%. Bone marrow examination revealed markedly decreased erythroid precursors and normal number and maturation of granulocytes and megakaryocytes. Two weeks later, the patient's platelet count decreased to 24,000/mm^3. She was transferred to our hospital. A second bone marrow examination revealed absent erythroid precursors and absent megakaryocytes. Granulocytic hyperplasia and multiple benign-appearing lymphoid aggregates were found. Bone marrow karyotype was normal. Flow cytometry of marrow aspirate

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revealed a population of T lymphocytes with a CD4/CD8 ratio of 0.63. There was no evidence of restricted clonality.

Physical examination was negative for lymphadenopathy and organomegally. A computed tomography (CT) scan of the thorax was normal. Tests for antinuclear antibodies, human immunodeficiency virus, and parvovirus B19 DNA IgM were negative. Hepatitis B surface antibody and hepatitis C antibody were positive. Parvovirus B19 DNA IgG was positive. After failing to respond to one course of intravenous immunoglobulin (2 g/kg over 2 days), cyclosporin A (CSA) (200 mg/day) and prednisone (60 mg/day) were begun. The CBC remained unchanged after 1 month of therapy. A third bone marrow examination revealed islands of myeloid cells and a marked decrease in lymphoid aggregates. Erythroid precursors were absent, and only rare megakaryocytes were seen. At this time, flow cytometry of marrow aspirate again revealed a population of T lymphocytes; however the CD4/CD8 ratio was 1.26. Again, there was no evidence of restricted clonality. The dose of CSA was increased to 400 mg/day.

After 2 months of CSA/prednisone, the patient demonstrated an increase in platelet count (69,000/mm^3), Hgb (8.7 gm%), and reticulocyte count (2.9%). Over the next 3–4 months, the prednisone was tapered off and CSA was continued. CBC revealed Hgb 13.1 gm%, platelets 139,000/mm^3, and WBC count 4,400/mm^3. One month after discontinuing prednisone, the platelet count decreased to 10,000/mm^3 and the Hgb decreased to 10.4 gm%. The WBC count remained normal. A fourth bone marrow examination revealed absence of erythroid precursors and megakaryocytes with normal myeloid maturation and numbers. The marrow again contained many benign-appearing lymphoid aggregates. Upon reinstitution of prednisone, the patient's platelet count and Hgb increased. However, while on CSA/prednisone, she developed severe thrombocytopenia, anemia, and mucosal bleeding. She became refractory to platelet transfusion.

The patient was given cyclophosphamide 750 mg/m^2 (day 1), vincristine 2 mg (day 1), and prednisone 60 mg/day × 5 days (CVP) every 4 weeks for six cycles. By the second cycle, there was a rise in Hgb and platelet count. After completion of six cycles, her Hgb was > 14 gm%, and her platelet count was in the range of 80–90,000/mm^3. She was placed on CSA (400 mg/day) as maintenance and this was subsequently tapered to 100 mg/day. As of the time of this report, 1 year since completing six cycles of CVP, her Hgb remains 14–15 gm% and her platelet count is 90–115,000/mm^3 (Figs. 1, 2).

During her remission following cyclophosphamide, vincristine, and prednisone (CVP) therapy, erythroid colony assays were performed on bone marrow and peripheral blood with and without the addition of serum collected at the time of initial presentation and at the time of remission. No inhibition of CFU-E and burst-forming units-erythroid (BFU-E) was observed to result from admixing her cells with her serum collected at the time of initial presentation or at the time of remission (Table I).

MATERIALS AND METHODS

After written, informed consent, 3 ml of bone marrow aspirate and 30 ml of peripheral blood were collected in separate preservative-free sodium heparin tubes.

Clonogenic Assays

Marrow aspirates were subjected to red blood cell (RBC) lysis in 4:1 buffered ammonium chloride solution, washed in Iscove's Dulbecco's modified Eagle's (DME) medium supplemented with 2% fetal bovine serum (2% FBS), and suspended in 2% FBS at a final concentration of 2×10^6 cells/ml.

Peripheral blood cells were washed once in Hank's balanced salt solution (HBSS), and light-density (mononuclear) cells were isolated by density-gradient centrifugation on a Ficoll-Hypague (1.077 g/ml) cushion (Histopague 1077, Sigma, St. Louis, MO), then washed in HBSS/0.1% bovine serum albumin, and resuspended in Iscove's DME medium to give a final concentration of 2×10^6 cells/ml.

Blood or marrow mononuclear cells were cultured in 35 mm dishes at a final concentration of 2×10^5 cells/dish in Iscove's methylcellulose medium (HC 4230, Stem Cell Technologies, Vancouver, Canada) supplemented with 9% agar-stimulated human leukocyte-conditioned medium HC 2300 (Stem Cell Technologies) and 2 U/ml erythropoietin (Amgen, Thousand Oaks, CA). The cultures were prepared with the patient's serum collected at initial presentation (serum A) or serum collected after the patient achieved remission (serum B). Cultures were incubated at 37°C in humidified 5% CO_2 atmosphere. Colonies were scored by standard morphologic criteria on day 14 [5].

DISCUSSION

We present a case of a 32-year-old woman with both erythrocytic and megakaryocytic aplasia with normal granulocytic maturation. A recent review of acquired pure red cell aplasia and acquired amegakaryocytic thrombocytopenia purpura has been provided elsewhere [6]. To our knowledge, one previous case has been reported. In that case, erythrocytic and megakaryocytic aplasia developed during pregnancy and slowly and gradually improved without treatment over $1\frac{1}{2}$ years after delivery [7]. The presentation, bone marrow pathology, response to therapy, and relapse pattern in our patient are not consistent with aplastic anemia. Most cases of amegakaryocytic thrombocytopenia or pure red cell aplasia are thought to

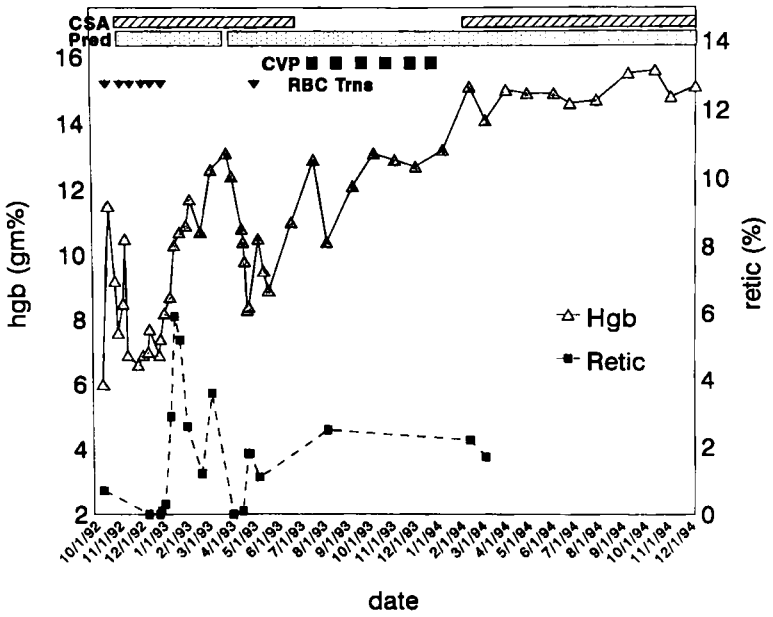

Fig. 1. Hemoglobin and reticulocyte count during the course of this patient's treatment. CSA, cyclosporine A; Pred, prednisone; CVP cyclophosphamide, vincristine, prednisone; RBC Trns, red blood cell transfusions; Hgb, hemoglobin; Retic, reticulocyte count.

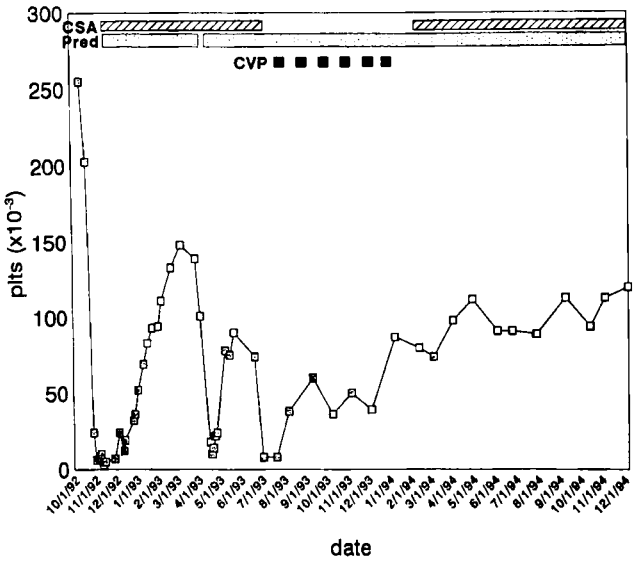

Fig. 2. Platelet count during the course of this patient's treatment. CSA, cyclosporin A; Pred, prednisone; CVP cyclophosphamide, vincristine, prednisone; plts, platelets.

be immune mediated, and evidence suggests both humoral and cell-mediated mechanisms [8–19]. The response to immunosuppressive agents and the presence of lymphoid aggregates strongly suggest an immune-mediated mechanism in our patient. There is also evidence suggesting the existence of a common bipotential stem cell for erythrocytes and megakaryocytes [2], as well as shared phenotypic expression of these two lineages [3,4,20]. Our case may thus represent immune-mediated damage or suppression of a precursor cell common to both lineages or an immune-mediated mechanism directed at a common antigen expressed on precursor cells of both lineages.

A recent hypothesis, based on hypoxia-induced thrombocytopenia, provides evidence that megakaryocytes and erythrocytes may share a common precursor cell [2]. In laboratory animals, hypoxia stimulates erythropoiesis, which induces thrombocytopenia. According to the "stem cell competition" hypothesis, thrombocytopenia here is believed to result from a reduction of platelet production secondary to the erythropoietin stimulation of erythropoiesis [2]. One clinical observation supports this notion: in cyanotic congenital heart disease, erythrocytosis is accompanied by thrombocytopenia [21].

Molecular and immunologic data supporting the association of erythroid and megakaryocytic cell lines include: 1) the identification of an erythroid nuclear factor important in the regulation of the transcription of globin specifically expressed in megakaryocyte lineage [3]; 2) a specific DNA-binding protein of the erythrocyte lineage identified in a human megakaryocyte cell line [4]; and 3) the recognition in normal bone marrows of a cell population that co-expresses megakaryocytic and erythrocytic markers in culture [20].

We cultured our patient's peripheral blood and bone marrow cells in an attempt to detect an effect of her serum on the growth of BFU-E and CFU-E. No inhibition of BFU-E and CFU-E was detected in the presence of her

TABLE I. Colony-Forming Cell Numbers In Patient's Bone Marrow and Peripheral Blood Cultured During Remission in Presence of Serum Collected at Initial Presentation (Serum A) and During Remission (Serum B)

	CFU-E	Mature BFU-E	Primitive BFU-E	CFU-GM	CFU-GEMM
Peripheral blood (per ml)					
Patient cells only	32	23	3	55.5	8.5
Patient cells/serum A	32	43.5	5.5	32	11.5
Patient cells/serum B	46.5	31.5	6	46.5	3
Bone marrow (per 2×10^5 nucleated cells)					
Patient cells only	29.5	11.5	2	28	1
Patient cells/serum A	31	10	1	17	2
Patient cells/serum B	30.5	7	2	46	3

CFU-E, colony-forming unit-erythroid; BFU-E, burst-forming unit-erythroid; CFU-GM, colony-forming unit-granulocyte, monocyte; CFU-GEMM, colony-forming unit-granulocyte, erythroid, monocyte, megakaryocyte; serum A, serum collected at time of initial presentation; serum B, serum collected during remission.

serum collected at the time of initial presentation (Table I), from which we can only speculate about the pathogenesis of her illness. This finding is consistent with a cell-mediated mechanism that may be dependent on the presence of activated suppressor T lymphocytes. It is of note that flow cytometry revealed the presence of T lymphocytes among the marrow cells that were correlated with disease activity. If an antibody was present, it may have been directed against an erythrocytic antigen expressed after the stage of CFU-E. Such an antigen would also have to be expressed on megakaryocytic progenitors and not on granulocytic progenitors. Cell surface antigen expression during early erythroid and myeloid differentiation has been described [22].

Glycophorin A is a candidate for such a red cell surface antigen expressed after the stage of CFU-E. This antigen is not expressed during myeloid differentiation [22]. Of further interest is the finding of a megakaryocyte cluster co-expressing glycoprotein IIIa (GPIIIa) and glycophorin A early in culture of normal bone marrows [20]. These findings, along with the patients' clinical presentation and our in vitro results, could suggest that an anti-glycophorin antibody is responsible for the mechanism of our patient's disease process. However, if an anti-glycophorin A antibody was present, one would expect the pre-transfusion antibody screen to be positive. Our patient's antibody screen was negative, excluding this possibility.

A cell-mediated immune mechanism is the most likely explanation for the pathogenesis of our patient's disease. When she first presented and upon relapse, her bone marrow contained an increase in lymphoid aggregates with a CD4/CD8 ratio of 0.63. One month after CSA and prednisone, there was megakaryocyte regeneration and a marked decrease in lymphoid aggregates with a CD4/CD8 ratio of 1.26. Cell-mediated immune mechanisms have been described in both pure red cell aplasia and amegakaryocytic thrombocytopenia [8,16–19]. The evidence in aplastic anemia is clearer that T lymphocytes, mainly CD8+ lymphocytes, may be involved in the pathogenesis [23]. Co-culture studies in aplastic anemia implicate activated suppressor T lymphocytes [24]. Our patient's bone marrow pathology, flow cytometry findings, and response to therapy suggest that local marrow infiltration with CD8+ T lymphocytes is the likely mechanism to explain her disease.

CONCLUSIONS

We have presented a patient with concurrent erythrocytic and megakaryocytic aplasia. Her bone marrow pathology, flow cytometry findings, and response to therapy, as well as our in vitro data, suggest a cell-mediated immune mechanism. It is of further interest that this case presents strong clinical evidence supporting the molecular, genetic, and immunologic data that a relationship between the erythrocytic and megakaryocytic cell lines exists. This relationship may consist of common phenotypic markers or a common bipotential stem cell.

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